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RESEARCH ARTICLE

ENHANCEMENT OF ANTIBIOTICS IN BIOSYSTEM BY NIOSOMAL DRUG DELVERY METHOD

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ABSTRACT

Therapeutics plays a vital role in this process for the treatment of diseases and the various ways of administering the drug in to the body. Niosome a vesicular drug carrier found its way in the field of pharmaceuticals. Bioavailability is attained when the drug is satisfied by all parameters which affect the activity of the drug like Adsorbtion, Distribution, Metabolism and Excretion. They are useful in carrying the drug to the target site, without any alteration in their composition and structure. The drug showed a very good activity on the microbial culture, there was also a good stability in the drug on pH. The drug was tested for all the parameters that affects the drug's activity and then only used for delivering. *In vitro* drug release studies were carried out in order to study the drug's release rate. The required quantity of the drug was delivered to the target site without any alteration of the drug (Ampicillin). Thus on the basis of studies conducted we can state that Niosomes possess great potential as drug delivery carriers orally.

Keywords: *Drug delivery, vesicles, therapy and noisome.*

INTRODUCTION

Therapy or treatment is the attempted remediation of a health problem, usually following a diagnosis that will be of great use to the mankind. Gene therapy is the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat disease. Usually viruses are used as vectors to carry the foreign gene into the host. A major drawback of virus based gene therapy is the immunogenicity of the vectors. Not only could the vector provoke an immediate adverse reaction to itself, immune protection can develop over time to the extent that repeated administration of the viral vector becomes useless. Also this gene therapy may trigger an oncogene. Chemotherapy in the simplest sense is the treatment of an ailment by chemicals. An alternative approach is the direct transfer of the non-defective bioactive gene products, peptides or proteins in their native conformation[1-2]. Although the transferred agents will not persist to the same degree as nucleic acid based vectors, they should be able to persist for a sufficient length of time to have efficacy in the transient therapeutic window. Delivery of therapeutic proteins to cells has certain advantages over delivery of nucleic acid. Since these proteins can be selected to carry correct post-translational modifications if any (e.g. glycosylation , phosphorylation) and can be of the same origin as the host, they will be well tolerated by the hosts and should not induce any immunogenic reaction[3-5].

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Improved patient compliance is due to; reduced frequency of administration and reduced side effects is due to low dose. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation[6].

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomal drug delivery system used to increase the bioavailability of the drug. Hence in the present investigation prepare Niosomes with higher entrapment efficiency and to check the stability of Niosomes in different temperature and pH.

MATERIALS AND METHODS

Solubility analysis: Five mg of ampicillin was dissolved in 10ml of distilled water. Initially the pH was 7.2 after the addition of ampicillin the pH was adjust at 4-5. Since the pH of ampicillin is 4-5. The proper vertox should be done for the dissolving of ampicillin. This was made as stock solution. The stock solution was taken in different concentrations along with the distilled water and made upto 3ml. Absorbance value was calculated using uv-spectrophotometer at 283nm.

Screening of antibiotics using ampicillin: 200ml of LB agar medium was prepared in conical flask and Petri plates were well packed . Both the LB agar medium and petri plates were autoclaved at 121^0 C for 15mins. After the autoclave , the medium was allow to cool in the laminar chamber . Then the medium was poured using pour plate method and the wells were made using gel puncture . The drug was taken into the wells at different concentrations . Plates were incubated at 37^0 C for 24 hrs. As a result , the zones were formed and its diameter was calculated .

Niosome preparation*:* Solution of surfactants/lipids is prepared by dissolving both in organic solvent (chloroform). The organic solvent is removed by rotary flask evaporation/under reduced pressure leads to formation of drug surfactant/lipid film. The surfactant/lipid

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film is then hydrated with aqueous solution of drug at temperature slightly above the phase transition temperature of surfactants used, for specified period of time (time of hydration) with constant mild shaking.

Niosome encapsulation of the drug hand shaking method: The vesicles in the round bottomed flask gets evaporated . 10 ml of drug sample was taken in a syringe .It was added to the round bottomed flask containing the vesicles . The addition of the drug was done by drop by drop and constant shaking in the water bath at 50^0 C .

Determination of entrapment efficiency: Niosomes containing drug were separated from unentrapped drug by centrifugation. This purified niosomal dispersion was used for further studies. The niosomal dispersions were centrifuged at 4500 rpm for 30 minutes and the decanted fluid was separated from the sediment material (the niosomes containing the entrapped drug). The separated niosomal suspension (1 ml) was disrupted using 3 ml 50% propanol for 5 min. which was then analyzed spectrophotometrically for drug concentration at λ_{max} 280 nm to calculate the amount of entrapped drug against 50% propanol as blank. The percentage of entrapped drug was calculated by applying the following equation.

% entrapment $=A_e\ x100\ /\ A_i$, Where, A_e is the amount of entrapped drug and A_i is the initial amount drug in the lipid phase.

Stability studies: The stability studies of the optimized niosomal formulation were performed at different conditions of temperature and pH and the action of enzymes and its effect on physical characteristics and drug content was noted.

pH stability: The niosomal dispersions (1 mg drug entrapped niosome /5ml of pH 7 phosphate buffer) were kept in the air tight containers and stored at five different pH 3, 5, 7, 9, 11 for 2 hours and the samples were withdrawn.The supernatant were analyzed spectrophotometrically at λ_{max} 280 nm after centrifugation with 4500 rpm for 30 minutes.

***In vitro* drug release:** A study was done on the release pattern of the drug from the niosomal formulations prepared by thin film hydration method. After seperating the unentrapped drug, the niosomal suspension containing drug equivalent to drug content was pipetted into the dialysis bag which was previously soaked and washed several times with distilled water. This was placed in 100 ml of phosphate buffer saline (pH 7.4) and kept with constant agitation on a magnetic stirrer maintaining a temperature of 37 C. Each periodical time the whole sample were withdrawn and same volume of fresh sample was replaced. Then the samples were assayed spectrophotometrically at 280 nm using medium as blank. The release was compared with pure drug solution.

RESULTS AND DISCUSSION

The development of a novel drug delivery system with great patience compliance to the pharmaceutical industry is of present need. Oral administration of drug would be the only way to achieve such patience compliance. Since oral delivery of drug will have many barriers to cross through, proper carrier is needed. Niosome would be such a carrier to surpass all problems that oral drug delivery confronts. To start with the standard values of Drug (Ampicillin) must be estimated to compare with later experimental values.

The niosomal formulations were prepared by varying concentrations of surfactant, cholesterol and drug by hand shaking method. The various ratios of all niosomal formulations were taken for size analysis. Measurement of the niosome size was made by using a optical microscope. Before to determine the size analysis, first we calculated the correction factor using stage micrometer and eye piece micrometer with microscope using 10 X magnification. Nice vesicular structures were seen. The vesicle size and size distribution values of various ratios of formulation were calculated using 45 X magnification. The diameter of niosomes was found to be in the range of 150-175 nm. To view it under still microscopic level, it was subjected to Scanning electron microscopy (Figure 1) to know the exact size and shape of niosomal formulations. Niosomes was found be mostly spherical and slightly elongated with size ranging around 165nm. The vesicles were discrete and separate with no aggregation or agglomeration.

Entrapment efficiency: The amount of drug entrapped was analyzed with standard entrapment efficiency method. The entrapment efficiency varied with different concentration of drug, solvent and surfactant.The entrapment showed around 55% in all formulations with highest of 59±2% in 1:1:1+10 formulation (Surfactant: Cholesterol: Drug + Diethyl ether). Since this entrapment will lead to good releasing of drug into the system, we went for *invitro* drug release.

In vitro drug release: The membrane permeability system in different time intervals was considered to check the *invitro* release of drug. The OD values measured was compared with the standard values to know the drug release. On estimating the drug release, the amount released seems to be high in first few minutes which decreased on proceeding time; until it reach the long time, its releasing increases gradually.

Stability Studies: In order to measure its stability in human system ; the drug entrapped Niosomes was checked for stability at different temperatures wherein it showed very less leakage of drug at typical body temperature and stable at storage temperatures. Further to know the stability of Niosomes in the gastro intestinal tract, it was subjected to various pH conditions (Table 3) and the result showed it released very well in pH ≥7. This makes a clear point that it will release drug only in basic environment passing all barriers of acidic conditions.

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Table 1: *In vitro* drug release analysis.

S.no	Samples	Time (mins)	O.D at 253nm
1	1	15	0.356
2	2	30	0.382
3	3	45	0.435
4	4	60	0.453
5	5	75	0.823
6	6	90	0.441
7	7	105	0.481
8	8	120	0.452
9	9	135	0.442
10	10	150	0.412

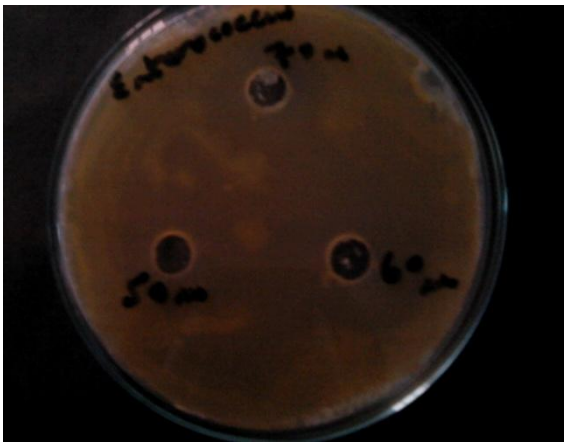
Table 2: *In vitro* drug release between Time vs O.D.

Time (mins)	Absorbance %
15	35.6
30	38.2
45	43.5
60	45.3
75	82.3
90	44.1
105	48.1
120	45.2
135	44.2
150	41.2

Table 3: pH stability of niosomes

pH	O.D. value (280nm)	Drug released(mg)
3	0.072	0.24
5	0.092	0.31
7	0.318	0.52
9	0.121	0.43
11	0.225	0.48

Figure 1: Activity of ampicillin on *Enterococcus.*



CONCLUSIONS

The pharmaceutical industry still suffers in need of proper delivery system for drugs. Normally oral drug delivery confronts a lot of problems like lesser bioavailability of drugs. The delivery systems currently available have least bit of patience compliance making them uncomfortable with side effects. This led to the development of Niosomes for carrying drug into the system orally overcoming the barriers of gastro-intestinal tract. The formulations were showing satisfactory particle size, encapsulation efficiency and invitro release studies. Niosomes offer advantages over other drug carriers with respect to ease of preparation, their capacity to carry large amount of drugs and biocompatibility. The results reveal that Niosomes are comparatively stable at storage temperature. The action of pH even did not show considerable effect on Niosomes and they release entrapped drug at basic pH. Thus on the basis of studies conducted we can state that Niosomes possess great potential as protein delivery carriers orally.

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